PRODUCTION, PARTIAL CHARACTERISATION AND BIOASSAY OF TOXINS FROM *PHYSALOSPORA TUCUMANENSIS* SPEG. - SUGARCANE RED-ROT FUNGUS

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ABSTRACT. – In the *in vitro* studies carried out on *Physalospora tucumanensis*, a phytotoxin was produced in liquid culture; this also induces red-rot symptoms on sugarcane, similar to that of the real fungus. Toxin production started from the 6th day of culturing and got to maximum at the 10th day. The toxin belongs to the anthraquinones, is soluble in water and most organic solvents, has a chromatography RF value of 0.78 and is yellow in colour. The lowest quantity that can cause plant reaction (red-rot) was 5 µg and it took at least 24 hours to initiate the symptoms.

RÉSUMÉ. – *Physalospora tucumanensis* produit une phytotoxine en milieu liquide. Celle-ci entraîne des symptômes de Morvet rouge sur la canne à sucre. Ils sont semblables à ceux produits par le champignon lui-même. La production de toxine est décelée à partir du 6ème jour de culture, et atteint son maximum au 10ème jour. La toxine est une anthraquinone, elle est soluble dans l'eau et dans la plupart des solvant organiques, elle présente une RF de 0,78 en chromatographie et est de couleur jaune. La quantité minimale permettant d'observer une réaction de la plante est de 5 µg et les symptômes sont observés après 24 heures d'incubation.

KEY WORDS : *Physalospora tucumanensis*, phytotoxins, anthraquinones.

INTRODUCTION

Physalospora tucumanensis Speg., the causal organism of the red-rot disease of sugarcane, has been assuming great importance in the country's sugar estates due to its severity on sugarcane varieties. There has been the general belief that resistant varieties can be regarded as the best solution to pathogenic diseases.

It has been established that some fungi associated with sugarcane, produce toxin : for example, *Fusarium moniliforme* Sheldon the causal organism of Pokka boeng of sugarcane (SINGH & SINGH, 1983), and *Helminthosporium sacchari* Butler the leaf spot of sugarcane pathogen (STEINER & BYTHER, 1971).

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Thus, there is the need to study toxin production of *P. tucumanensis*, in order to provide *in vitro* screening methods which will give quicker results in testing sugarcane varieties for resistance.

MATERIALS AND METHODS

The red-rot diseased cane stalk was cut into bits, surface sterilised with 0.1 % mercuric chloride for 1 mn and plated on potato dextrose agar (PDA). After the 4th day, the fungus growing on the PDA was subcultured on to fresh PDA plates until pure culture of *P. tucumanensis* was obtained.

Toxin was produced in still cultures at 27°C in 250 ml Erlenmeyer flasks, each containing 50 ml of Fries medium (MACRI & VIANELLO, 1976). Each flask was inoculated with a small agar block from a 10 days old sporulating culture of the fungus. Samples were taken every 3 days up to the 15th day, during which the liquid culture was filtered through sterile filter paper and the filtrate reduced to 1/20th of its original volume by evaporation *in vacuo* at 45°C. The residue was mixed with equal volume of methanol and well agitated. The content was again evaporated *in vacuo* at 45°C leaving a straw yellow substance. This was redissolved in methanol and run on silica gel thin layer chromatography using ethyl acetate - methanol as the running solvent. The band formed was redissolved in methanol and re-evaporated to leave brown yellow crystals.

A little quantity of the substance was dissolved in methanol and run on Pye-Unican ultraviolet spectrophotometer model Sp 1800, while another portion of the same was also run on Pye-Unicam infra-red spectrophotometer. The toxin was dissolved in water, ethanol, acetone, chloroform, and methanol to detect its solubility.

Bioassay with sugarcane stem

Serial dilutions of 10^{-1}, 10^{-2} to 10^{-6} of the substance were made using water (10^{-1} = 1 g/10 ml to 10^{-6} = 1 µg/ml of toxin solution).

Half ml of each dilution was poured on to the inner part of half split sugarcane stalk (5 cm long) and closed up, covered with aluminium foil and incubated at 27°C. Five replicates were made per treatment, per dilution, and five of these set up were made to allow for enough samples of 5 days at 24 hours intervals.

Bioassay with sugarcane leaves

With the aid of sterile microsyringe, 0.05 ml of each of the dilution was injected in the petiole of 2 months old sugarcane leaf : one set of tests was carried out on intact leaf on the plant while another set was applied on to detached leaves and incubated at 100 % relative humidity and 27°C. The inoculated leaves were examined every 24 hours for possible symptoms development.

RESULTS

The toxin was produced from the 6th day after inoculation. However, there was no significant difference from the quality of toxin obtained from 12 to 15 days after inoculation.

The toxin was soluble in water, ethanol, methanol, acetone, but very little in chloroform. It was thermostable and did not show any change in activity when autoclaved for 30 mn at 1.1 kg/cm^2 and 121°C. The toxin travelled as a single spot RF 0.78 in the thin layer chromatography (solvent : ethyl acetate-methanol (50/50, v/v) using silical gel plates treated with 0.01 M NaOH).

Characterisation

The isolated toxin has peaks at 300, 264, 225 and 210 nm in the ultraviolet region. This is similar to that observed for some anthraquinones such as emodin and chrysophanol (HARBONE, 1973). Like emodin, the carboxyl (γ-COO) stretching frequency in the infra red occurs at 1630 cm^{-1}, indicating that the carboxyl group is chelated (HARBONE, 1973).

Table 1 – Reactions of stem and leaves of sugarcane to *Physalospora tucumanensis* toxin after 24 hrs of incubation.

Tableau 1 – Réactions de la tige et des feuilles de la canne à sucre à la toxine du *Physalospora tucumanensis* après 24 heures d'incubation.

Toxin solution	serial dilutions					
Volume	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Stem : 0.05 ml	+	+	+	+	+	–
Leaves : 0.05 ml	+	+	+	+	–	–

Nota : + = plant reaction, – = no plant reaction.

From the dilution series and the volume of toxin solution used, it was observed that 5 μg was the lowest quantity for stem as well as the leaf assay for symptoms initiation (Table 1). The characteristics red-rot symtoms was obtained in both the stem ans leaf midrib after 24 hrs of assay with the toxin. The inability of the toxin to react with the following plant species : *Sorghum vulgare* L., *Zea mays* L., *Pennisetum purpureum* L., *Cynodon nlemfuensis* Vanderyst and *Oryza sativa* Lf., showed that it is host specific.

DISCUSSION

It has now been established that *P. tucumanensis* produces a host specific phytotoxin in still liquid culture.

However, the bioassay conformed with the work of MACRI & VIANELLO (1976) on *Curvularia lunata* (Wakk) Boed. Symptoms obtained by inoculation

with the fungi were similar to those obtained with the toxin in the *in vitro* experiment (WHEELER & LUKE, 1963). The results showed that for any concentration, even about the phytotoxic level, it would take at least 24 hours for the toxin to diffuse into the plant cells and cause reaction. The lowest amount of toxin obtained in this study which could cause plant reaction is about 5 μg, which is calculated from the serial dilution concentrations.

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